Supporting information for

Single virus-sensitive barcode PCR by aggregation of gold nanoparticle probes

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Construction of HA Plasmid

To construct the plasmid containing HA gene of H3N2 virus (HA plasmid)， the HA gene were amplified by PCR (primers were showed in Table S1). The amplification product and pET-28a (+) plasmid were simultaneously processed with double enzyme digestion, followed by ligation at 16°C overnight. Competent cell was melted on ice, followed by the addition of the recombinant plasmid. The reaction was kept on ice bath for 30 min, and then transferred to 42°C water bath for 30 s, followed by ice bath for 2 min. LB liquid medium was then added to the mixture, followed by mixing in an oscillator with 220 rpm at 37°C for 1 h. The transformed product was cultured on an LB estimation medium with 30 μg/mL of Kanamycin. 7 single clones were selected for PCR identification, followed by positive clones screen for sequencing. Positive clones were cultivated in LB liquid medium containing Kanamycin at 37°C and 220 rpm overnight, followed by the extraction of the HA plasmids. Finally, the quantified HA plasmids were stored at -20°C until use.

Table S1. Primers for HA plasmid.

Primer	Sequence (5' to 3')
HA-Xhol	ccgctcgagATGGAGACAGTATCACTAATAACTA
HA-Notl	ataagaatgcggccgcTTATATACAAATGTTGCATCTGCAAGA

Preparation of GNP probes

The SH-DNA primers including six capture DNAs that were designed to bind to different segments of HA gene of H3N2, the capture DNAs and barcode DNA (their sequences can be seen in Table S2) were diluted to 100 nM in eppendorf tubes, respectively, and then stored at -20°C prior to use. Firstly, 100 μL of each of 6 types of capture DNAs with a concentration of 100 nM, 100 μL of the barcode DNA with a concentration of 10 nM, were mixtured with 100 μL of 1 nM of 15 nm GNP (the concentration of GNP has been determined by the supplier or us) at 25 °C for 10 min, and placed at -80°C till completely frozen. The mixture was first centrifuged at 4 °C, 12,000 g for 10 min after thawing, and the supernatant was discarded and the GNP precipation was washed with 100 μL of PBS (10 mM, pH 7.2). The washing process was repeated three times for each GNP probe, therefore the resulting six GNP probes in PBS were obtained and stored at 4 °C prior to use. The concentration of the successful prepared GNP probes were measured by UV-Vis spectrometer at a certain absorption peak of 450 nm and comparing with the 15 nm GNP.

***The standard GNP concentration was determined by the constant ε (molar extinction coefficient) of 15 nm GNP at 450 nm is 2.7×10^8 M^{-1} cm^{-1}) and the absorption value at 450 nm.

Number calculation of Barcode DNA coating each GNP probe

The concentration of barcode DNA immobilized on 100 μL of GNP probes was quantified by qPCR method;

The concentration of the prepared GNP probes can be determined by the ratio of the absorption peak values of GNP probes at 450 nm to that of 15 nm GNP. According to the their absorption peak values at 450 nm, the ratio is 0.382/0.464=0.823. Therefore, the concentration of GNP probe is 0.706 nM (4.25×10^8 particles/μL).

To get the concentration value of barcode DNA on GNP probes, we firstly drew the standard curve of qPCR method for the barcode DNA samples at different concentrations. Briefly, the barcode DNA probe (10 μM) was diluted by a 10-fold gradient to obtain five samples. 1 μL of each of samples was tranferred into qPCR reaction to get the corresponding Ct value using the primers (barcode-F: AAGGCCTTTGTCGGTAGCTC /barcode-R: AAAGGTGCCAACTTCATCCTCAT). As a result, we got 5 pairs of values (log value of barcode DNA concentrations vs. Ct value), which were plotted on the axis and fixed by a straight line to obtain the Figure S1. Then 1 μL of GNP probe was quantified by qPCR with the barcode DNA as the template and the Ct value is 9.35. According to the standard equation (Y= -3.505X + 35.82) in Figure S1, the log value of barcode DNA concentration of GNP probes is 2.15×10^9 copies/μL. Since the concentration of GNP probe is 4.25×10^8 particles/μL, therefore, the average barcode DNA amount is 5.06 ($2.15\times10^9/4.25\times10^8$) for each GNP.

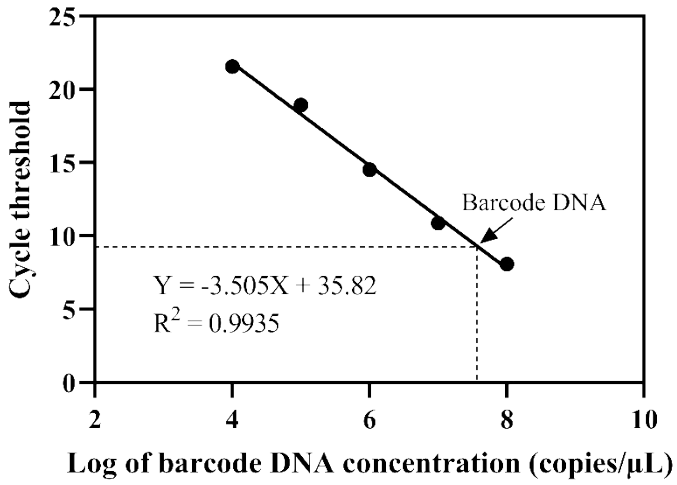

Figure S1. Number computation of DNA on optimal capture efficiency probe.

Plot of Ct values obtained using qPCR method accurately quantify the maximum linear range of barcode DNA concentrations.

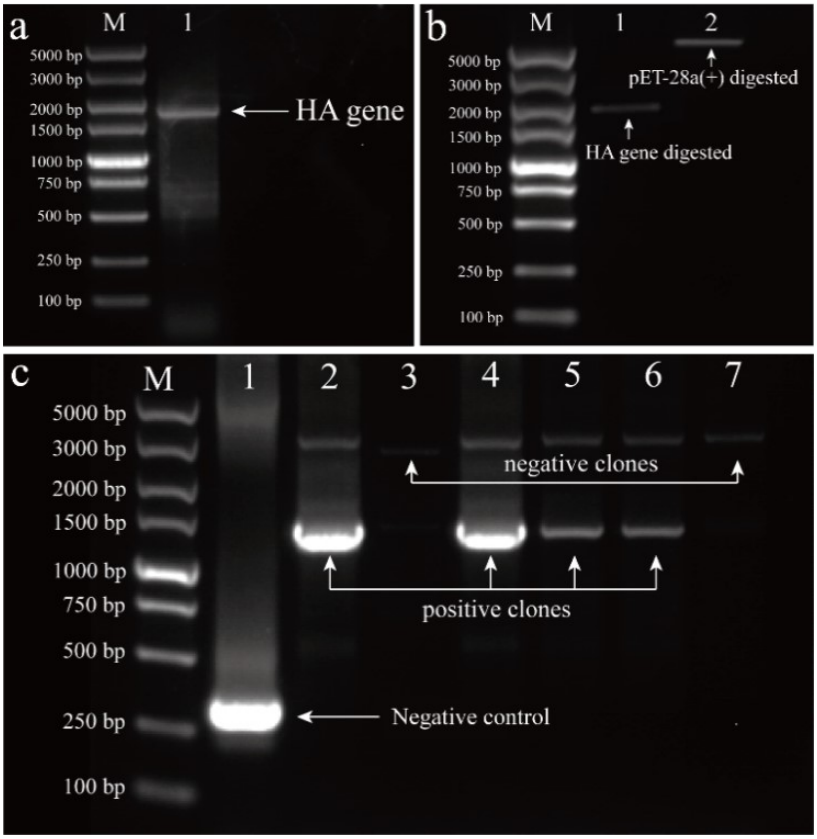

Figure S2. Verification of HA Plasmid.

(a) M: Vazyme DL5000 Marker. Lane 1: HA gene amplification product; (b) M: Vazyme DL5000 Marker. Lane 1: HA gene amplification product after double enzyme digestion. Lane 2: pET-28a (+) plasmid with double enzyme digestion; (c) M: Vazyme DL5000 Marker. Lane 1: negative control. Lane 2, 4, 5 and 6: positive clones of HA plasmid. Land 3 and 7: negative clones.

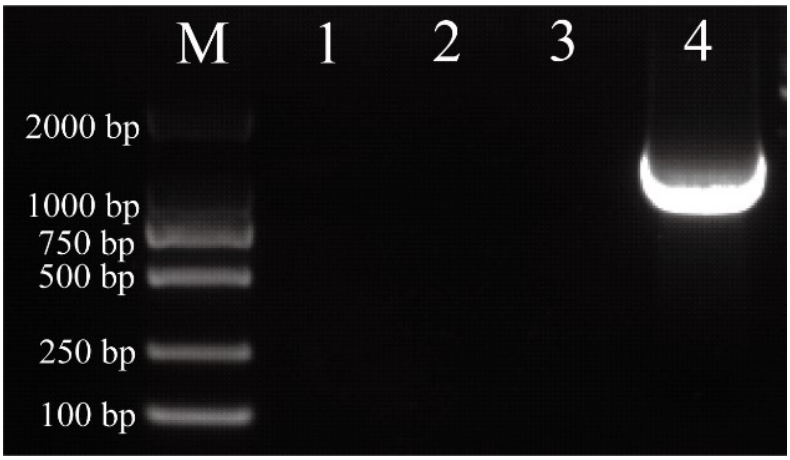

Figure S3. Verification of the GNP probe capture specificity.

M: Takara DL2000 Marker. Lane 1: negative control; Lane 2: H3N2 viral RNA; Lane 3: H9N2 viral RNA hybridized with 6 GNP probes; Land 4: H3N2 viral RNA hybridized with 6 GNP probes. For each sample, the precipitate was collected by centrifugation for PCR.

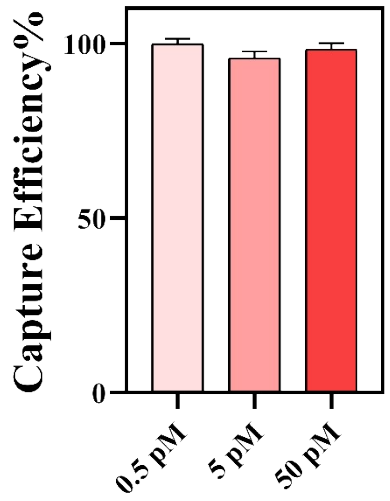

Figure S4. Capture efficiency of GNP probes with different concentrations.

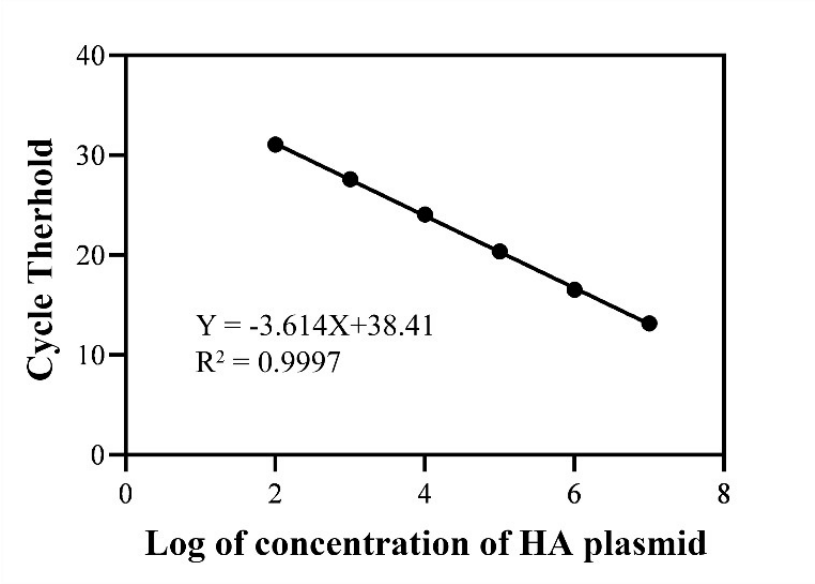

Figure S5. Standard curve of the HA plasmid by qPCR method.

Plot of Ct values obtained using qPCR method accurately quantify the maximum linear range of HA gene concentrations.

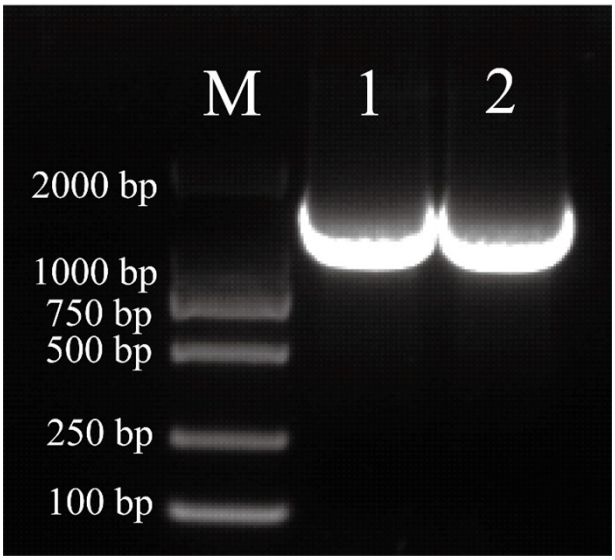

Figure S6. Verification of the stability of mixture of GNP probe and H3N2 viral RNA.

M: Takara DL2000 Marker. Lane 1: Amplification of H3N2 viral RNA; Lane 2: Amplification of H3N2 viral RNA hybridized with GNP probes.

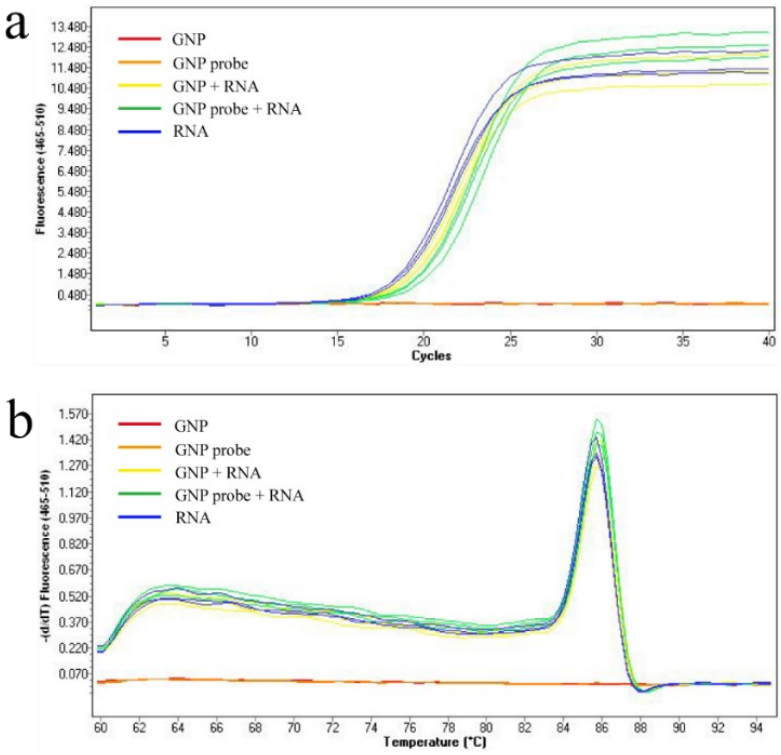

Figure S7. Interference test of GNP and GNP probe on RT-qPCR.

(a) RT-qPCR amplification curves. (b) RT-qPCR melting curves.

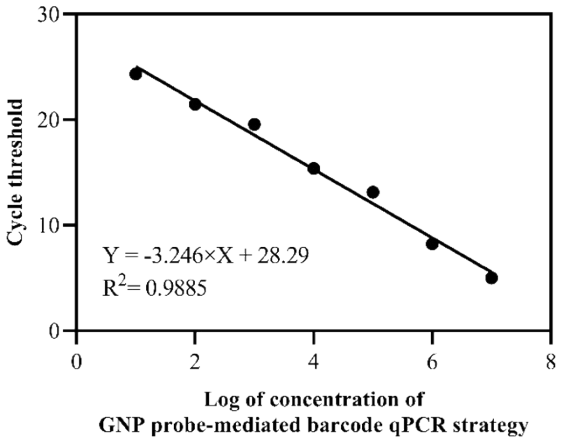

Figure S8. Standard curve of GNP probe-mediated qPCR strategy.

Plot of Ct values obtained using GNP probe-mediated qPCR strategy of minimum concentration required to reach a positive signal for HA gene.

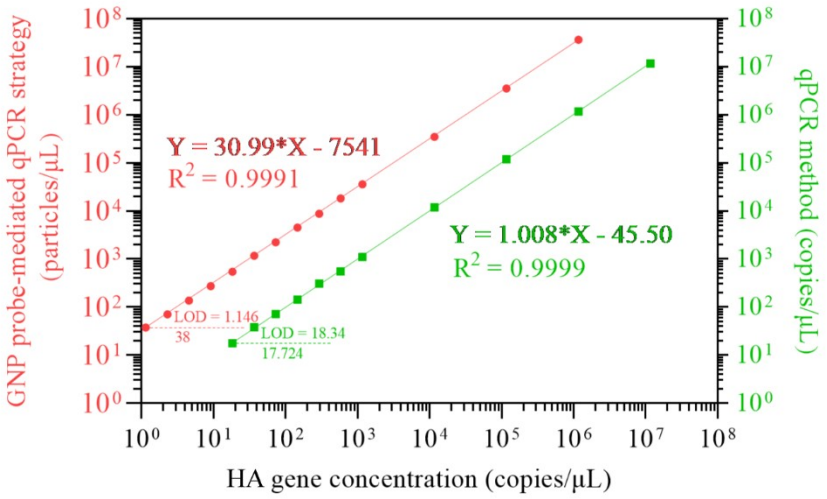

Figure S9. Calibration curves of GNP-mediated qPCR strategy and qPCR method.

The LOD is the intersection of the calibration curve with three times signal-to-noise ratio.

Table S2. Thiol Probes for Preparation 15 nm GNP Probes.

Probe	Sequence (5' to 3')
Capture DNA 1	SH-AAAAAAAAAAGAAGGTCTGGAGCCCAT
Capture DNA 2	SH-AAAAAAAAAATTCGCACATGCCTCCT
Capture DNA 3	SH-AAAAAAAAAAGATGTTGGCAGCATTTTGTT
Capture DNA 4	SH-AAAAAAAAAACGGGTGAAGTGATTTGTGG
Capture DNA 5	SH-AAAAAAAAAACAAGTAGGTGGCACGC
Capture DNA 6	SH-AAAAAAAAAACGCCTTCGGTCTTTGAGT
Barcode DNA	AAGGCCTTTGTCGGTAGCTCCAACACCTCCTCCTTCACCCCCGTCTCTATT GATGAGGATGAAGTTGGCACCTTTAAAAAAAAAA-SH

[1]SH- represents for thiol group.

Table S3. Capture efficiency of GNP probe with different concentrations.

H3N2 viral RNA	**Concentration (copies/μL)**	3.01×10^5	3.01×10^6	3.01×10^7
Barcode DNA	**Concentration (copies/μL)**	2.996×10^5	2.882×10^6	2.293×10^7
	CT values	18.77	15.21	11.96

Table S4. Ct values of H3N2 viral RNA diluted samples by qPCR method.

Concentration (copies/μL)	1.174×10^3	5.87×10^2	2.935×10^2	1.468×10^2	7.338×10^1	3.669×10^1
Ct values	27.58	28.67	29.6	30.75	31.86	32.83
Concentration (copies/μL)	1.834×10^1	9.172×10^0	4.586×10^0	2.293×10^0	1.146×10^0	Blank
Ct values	34.03	>35	>35	>35	>35	>35

Table S5. Ct values of interference test of GNP and GNP probe by RT-qPCR.

Sample Name	GNP	GNP probe	GNP + RNA	GNP probe + RNA	RNA
Ct values	-	-	18.88 ± 0.14	19.57 ± 0.27	18.36 ± 0.09

Table S6. Ct values of H3N2 viral RNA diluted samples by GNP-mediated qPCR Strategy.

Concentration (copies/μL)	1.174×10^3	5.87×10^2	2.935×10^2	1.468×10^2	7.338×10^1	3.669×10^1
Ct values	18.52 ± 0.24	19.39 ± 0.11	20.45 ± 0.33	21.38 ± 0.26	22.35 ± 0.34	23.38 ± 0.22
Concentration (copies/μL)	1.834×10^1	9.172×10^0	4.586×10^0	2.293×10^0	1.146×10^0	Blank
Ct values	24.34 ± 0.15	25.33 ± 0.15	26.29 ± 0.21	27.23 ± 0.24	28.11 ± 0.31	>35

Table S7. Concentrations calculated by Ct values of H3N2 clinical samples by qPCR method

Sample No.	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
Ct values	21.83	-	-	30.07	-	26.7	-	-
Concentration (copies/μL)	4.273×10^4	-	-	2.242×10^2	-	1.919×10^3	-	-
Sample No.	No.9	No.10	No.11	No.12	No.13	No.14	No.15	
Ct values	25.58	-	-	-	26.21	-	-	
Concentration (copies/μL)	3.918×10^3	-	-	-	2.623×10^3	-	-	

Table S8. Concentrations and Ct values of H3N2 clinical samples by GNP-mediated qPCR Strategy.

Sample No.	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
Ct values	13.37			20.82		17.77		
Concentration (copies/μL)	4.359×10^{4}			2.209×10^{2}		1.923×10^{3}		
Sample No.	No.9	No.10	No.11	No.12	No.13	No.14	No.15	
Ct values	16.85	25.22			17.29		26.86	
Concentration (copies/μL)	3.692×10^{3}	9.744×10^{0}			2.702×10^{3}		3.044×10^{0}	